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On the validity of the ^{13}C-acetate breath test for comparing gastric emptying of different liquid test meals: A validation study using Magnetic Resonance Imaging (MRI)

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Running title: ^{13}C-breath test validation by MRI

Abstract

Background:

^{13}C-acetate breath testing (BT) is applied to assess and compare gastric emptying of liquid meals. Gastric half-emptying times (t_{50}) from BT show offsets compared to t_{50} values from γ-scintigraphy and ultrasonography. Linear transformations have been proposed to correct these offsets. This investigation critically validates the BT for the assessment of liquid gastric emptying by using simultaneously recorded meal and total gastric content volume emptying data from MRI.

Methods:

Data were collected during a recently published double-blind, randomized, cross-over MRI gastric emptying study of three ^{13}C-labelled enteral formulas differing in protein sources (PMID:24699556). BT derived t_{50} was computed with the analysis methods commonly applied in gastric emptying research, i.e. the exponential-beta function and the Wagner-Nelson (WN) method, respectively.

Key Results:

BT t_{50} values from exponential-beta function and WN method showed a positive and negative offset to MRI data, respectively. Linear regression detected low concordance between MRI and both BT methods revealing meal specific and emptying rate dependent offsets. The WN method showed worse agreement and correlation with MRI emptying data. BT rather reflected meal volume than total gastric content volume emptying.

Conclusions&Inferences:

This validation study indicates that the ^{13}C-acetate breath test may not be applied to compare gastric emptying of arbitrary liquid meals without prior validation by imaging methods. t_{50} values from BT are biased by 1) the properties of the meal and 2) the selected method used for $^{13}CO_2$ exhalation analysis. No linear transformation common for all meals was applicable to correct the offsets between BT and MRI.

Keywords:

^{13}C-acetate breath test; gastric emptying; test meal; Wagner-Nelson method

Abbreviations:

BT: breath testing

cPDR: cumulative PDR

ExpBeta: exponential-beta function

FR: Fresubin Energy®

GCV: gastric content volume

MRI: magnetic resonance imaging

MV: meal volume

NU: Nutrison Energy®

OS: Osmolite HiCal®

PDR: percentage dose of ^{13}C recovered

t_{50}: half-emptying time

WN: Wagner-Nelson method

Key Messages

- Liquid meal half-emptying times (t_{50}) measured by ^{13}C-acetate breath testing have been reported to show offsets compared to values from γ-scintigraphy and ultrasonography
- Here, MRI was applied to systematically validate the ^{13}C-acetate breath test for the assessment of liquid meal and total gastric content emptying in three isovolumetric and isocaloric ingested enteral formulas.
- No meal independent linear transformation could be identified to achieve congruency between the ^{13}C-acetate breath test and the imaging modality.
- Meal and emptying rate dependent offsets were observed for the ^{13}C-acetate breath test indicating the need for prior validation by imaging methods when investigating gastric emptying of novel meal compositions.

Introduction

^{13}C breath testing has been extensively applied to study gastric emptying due to its non-invasiveness, cost-effectiveness and high availability. Meal labelling by ^{13}C-acetate is commonly used for the assessment of liquid gastric emptying. Different non-linear mathematical formulas were developed to extract the meal half-emptying time (t_{50}) from $^{13}CO_2$ exhalation data.(1, 2) Braden et al proposed to fit the $^{13}CO_2$ data by an exponential-beta (*ExpBeta*) function and Sanaka et al. presented an adapted Wagner-Nelson (*WN*) method for this purpose. The t_{50} values from both analyses, however, showed offsets towards γ-scintigraphy.(1-3) Validation studies using ultrasonography also showed offsets in the t_{50} values computed with the ExpBeta and the WN method, respectively.(4, 5) The offsets were assigned to ^{13}C-metabolism.(6-10) Corrections by a linear transformation were proposed to achieve congruency with the imaging modalities.(1, 5, 11)

Available ^{13}C breath test marker for gastric emptying have different affinities towards the liquid or solid meal phase.(11) Potentially, the exact composition and different chemical properties during gastric digestion of the test meal might influence the breath test marker emptying dynamics. This might distort results from ^{13}C breath test studies comparing different test meals.

Magnetic Resonance Imaging (MRI) has recently been validated for the assessment of gastric motor function.(12-15) MRI allows to visualize the volume and distribution of the ingested meal as well as of gastric secretion. Therefore, MRI represents an ideal modality for the validation of the ^{13}C-acetate breath test for the measurement of liquid gastric emptying.

This investigation aimed to validate the ^{13}C-acetate breath test for the comparison of liquid gastric emptying by means of MRI as the reference method. MRI derived volume data of meal and total gastric content (meal + secretion) from a recent gastric emptying study were used.(16) The recent study compared three ^{13}C-labelled enteral formulas that differed mainly in their type of protein composition, being either coagulating or non-coagulating in the acidic gastric environment. This investigation analyzed the $^{13}CO_2$ exhalation data by the *ExpBeta* and *WN* methods and compared it to the MRI data.

Methods

Ethics

The gastric emptying study was carried out according to Good Clinical Practice and the Declaration of Helsinki. The study protocol was approved on 18.07.2011 by the Ethics Committee Zurich (KEK-ZH-No 2011-0152/1) and registered at the Netherlands Trial Register (trial number NTR2979). All participants provided written informed consent.

Study design and test meals

This investigation used data acquired in a recently published gastric emptying study.(16) Therefore, the study design is described only briefly. The trial was performed in a single-centre double-blind randomized three-way cross-over design. 21 participants received three enteral formulas with similar amounts of fat, carbohydrates and proteins differing in protein sources (ref. **table 1**).

300 ml of the enteral formulas (450 kcal) were infused at 37°C via a nasogastric tube within maximum 5 minutes. For breath testing (BT), the enteral formulas were labelled with 100 mg of [1-^{13}C] sodium acetate (Euriso-Top, Saint-Aubin Cedex, France). Breath samples were taken before and every 10 minutes after infusion over a period of 180 minutes. MRI scans were performed at regular time intervals after infusion over a period of 185 minutes.

^{13}C-Acetate breath test

Breath samples were collected into aluminized bags. $^{13}CO_2$ $^{12}CO_2^{-1}$ ratios in the samples were determined by non-dispersive isotope selective infrared spectroscopy (IRIS® 3 Lab, serial number 005091, Wagner Analysen Technik GmbH, Bremen, Germany). The results were expressed as delta (δ) value per mil and delta over baseline ($DOB_t = \delta_{Sample} - \delta_0$). DOB_t was used to determine the percentage dose of ^{13}C recovered (PDR [% h^{-1}]) and the cumulative PDR (cPDR [%]) at each measurement time point until the end of analysis period.(11, 17, 18) The resulting PDR curves for each participant and nutrition solution are displayed in supplementary **figure S1**. Non-linear fits were performed using function nlme in program R, version 2.13.1 (R Foundation for Statistical Computing, Vienna, Austria).(19)

BT derived liquid meal emptying was quantified by computing t_{50} with the analyses described by Braden et al. (*ExpBeta* function) and Sanaka et al. (*WN* method) (1, 2, 11) resulting in the parameters BT_t_{50}_ExpBeta and BT_t_{50}_WN, respectively. The applied equations were:

$$\mathrm{BT\_t_{50}\_ExpBeta} = (-1 * k^{-1}) * \ln\left(1 - 2^{\left(-1*\beta^{-1}\right)}\right) \quad [1]$$

$$0.5 = [\mathrm{cPDR} * \mathrm{BT\_t_{50}\_WN} * \mathrm{cPDR}\infty^{-1}] + [-1 * k^{-1} * \mathrm{PDR} * \mathrm{BT\_t_{50}\_WN} * \mathrm{cPDR}\infty^{-1}] \quad [2]$$

The parameters *k* and *β* were derived from the fitted PDR curves.

Magnetic resonance imaging

Magnetic resonance imaging measurements were performed in the right decubitus on a 1.5 T whole-body MRI system (1.5 T Achieva; Philips Medical Systems, Best, The Netherlands) using an abdominal, four-channel phased-array receive coil for signal detection. Gastric content volume was assessed by a standard gradient echo sequence consisting of 30 image slices acquired during a single breath hold (~16 seconds) covering the entire upper abdomen.(16) T1 relaxation times of gastric content were imaged using a recently developed abdominal T1 mapping sequence (GOLD) performed during free breathing and consisting of 18 to 20 coronal image slices (~ 2.5 minutes).(12)

Gastric content volume (GCV) data, as presented in Kuyumcu et al.(16), was used to extract meal volume (MV) by subtracting secretion volume using the combination of the standard gradient echo sequence and T1 mapping MRI data according to previously reported procedures.(13, 15) The resulting MV curves (ref. supplementary **figure S2**) were used to compute the MRI derived meal-half emptying time MRI_t_{50}_MV by a simple exponential gastric emptying function, i.e. $MV(t) = MV_0 {*} e^{-(t/tempt)}$ and MRI_t_{50}_MV = $t_{empt}{*}\ln(2)$. Half-emptying time of GCV, MRI_t_{50}_GCV, was derived as published previously.(16)

Data analyses

Data plots and statistical analyses were done by GraphPad Prism® 6.01 (GraphPad Software, Inc., La Jolla, USA) and program R (R Core Team, R: A Language and Environment for Statistical Computing. 2015, R Foundation for Statistical Computing: Vienna, Austria). Agreement between MRI (MRI_t_{50}_MV and MRI_t_{50}_GCV) and breath test (BT_t_{50}_ExpBeta and BT_t_{50}_WN) was visualized by Bland-Altman plots (20) and quantified by Lin's concordance correlation coefficient (r_c).(21) r_c is the concordance between a standard measurement and another method. It quantifies the agreement between two measures of the same variable, i.e. the degree to which pairs fall on the identity line (45°-line), and can range from -1 to 1, with perfect agreement at 1. In addition, Pearson's correlation coefficient (r) was listed. The corresponding t_{50} values were correlated for each enteral nutrition separately. Values are given as mean difference or estimate with 95% confidence interval (95%CI).

Results

The final analysis included data of 20 volunteers for FR, 21 volunteers for NU and 18 volunteers for OS (ref. supplementary **figures S1 and S2**) with a mean age of 25 (range 21 to 40) years and a mean BMI of 21.6 (range 18.4 to 24.4) kg*m^{-2}.(16)

Bland-Altman plots of breath test and MRI derived t_{50}

For all enteral formulas, the *ExpBeta* function (BT_t_{50}_ExpBeta) overestimated t_{50} (**figure 1A** and **1B**), corresponding to a significant positive bias in the Bland-Altman plots (ref. **table 2**). This positive bias was more pronounced for the MV than for the GCV data. The overlapping 95% CIs of the biases (i.e., mean offsets) listed in **table 2** indicate comparable offsets for all the three enteral formulas. The *WN* method (BT_t_{50}_WN) underestimated t_{50} compared to MRI_t_{50}_MV and MRI_t_{50}_GCV (ref. **figure 1C** and **1D**). The corresponding significant negative bias was more pronounced for GCV (ref. **table 2**). Again, offsets were comparable for all three enteral formulas. A larger variability between total gastric content volume emptying and breath test data was indicated by wider limits of agreement. As depicted in **figure 1C** and **1D**, the deviation in measured t_{50} increased linearly for higher t_{50} values. This suggested that BT_t_{50}_WN values showed a t_{50} dependent offset compared to MRI data. Such linear deviation was not observed for BT_t_{50}_ExpBeta (**figure 1A** and **1B**).

Concordance of breath test and MRI data

The linear regression analysis confirmed the findings in the Bland-Altman plots. Lin's concordance coefficients (r_c) showed an overall low concordance with emptying rate dependent deviations of the ^{13}C-breath test and MRI data. These deviations were dependent on the BT analysis method and test meal. **Table 3** lists the calculated intercepts and slopes of the regression lines together with r_c and r and the respective

95% CIs. A low method concordance of ^{13}C-breath test and MRI data is evident when the regression line is shifted and tilted away from the identity line (i.e., 45°-line). (ref. **figure 2** and **table 3**). Lin's concordance and Pearson's correlation coefficients were larger for the *ExpBeta* function than the *WN* method. Although GCV regression lines for the *ExpBeta* function showed a strong tilt, i.e. regression slopes << 1.0, concordance was generally better (i.e., r_c was higher) for GCV as more t_{50} values coincided with the identity line. Only between BT_t_{50}_ExpBeta and MRI_t_{50}_MV for the enteral formulas OS and NU no t_{50} dependent change in the offset (no tilt in the regression line) was detected. However, a significant bias (i.e., shift away from the identity line) was evident resulting in a low method agreement (i.e., lower r_c).

Discussion

This validation study demonstrates that t_{50} values from ^{13}C-acetate breath testing are affected by 1) the properties of the liquid test meal and 2) the selected method used for $^{13}CO_2$ exhalation data analysis. Neither of the two commonly applied BT analysis methods, i.e. the exponential-beta function (1, 11) nor the Wagner-Nelson method (2, 4), produced t_{50} values that were in concordance to MRI derived values of meal or total gastric content volume emptying. Either an offset or both an offset and an emptying rate dependent deviation from the MRI t_{50} values was observed.

Meal specific influences on emptying rate dependent offsets in t_{50} between breath test and MRI

It is commonly understood that gastric emptying depends on the ingested nutrients, caloric content, volume and the consistency of the test meal.(22-24) Under this premise, the detection of differences between meals investigated by ^{13}C-breath test gastric emptying studies have always been assigned to the differentness of the investigated meals.(25-30) The results of the presented study indicate that also biases originating from the applied method and/or the properties of the test meal might lead to differences in ^{13}C-breath test measured half-emptying times. The comparison to MRI reference data using the method of Bland-Altman and the regression analysis revealed an emptying rate dependent and meal specific offset of the t_{50} values derived by the ^{13}C-acetate breath test. The main difference between the test meals was the protein source. The enteral formula NU contained little casein and was shown to remain liquid during *in vitro* digestion with gastric juice.(31) The two other, casein dominant enteral formulas, coagulated *in vitro*. It can be hypothesized that whether or not the test meal remains liquid during the course of gastric digestion plays a role in the distribution of the ^{13}C-marker throughout the gastric cavity, its subsequent emptying into the duodenum and absorption from there. This hypothesis was somewhat supported by the results of the *WN* method that showed highest concordance and correlation for the non-coagulating enteral formula. However, such differences in correlations were not observed with the *ExpBeta* function, suggesting a more complex cause of meal specific offsets in t_{50} from the ^{13}C-breath test.

Impact of breath test analysis method on emptying rate dependent offsets

The commonly applied *ExpBeta* function and *WN* method were used to extract t_{50} values from the $^{13}CO_2$ exhalation data. Both analysis methods for ^{13}C-acetate breath testing have been previously validated with γ-scintigraphy.(1-3) A t_{50} dependent offset in the regression line (intercept = 49 min, slope = 1.15), but also a good correlation of r = 0.95 was reported for the *ExpBeta* function.(1) The *WN* method underestimated the t_{50} values from γ-scintigraphy, however, was claimed to make “the results of the ^{13}C breath tests comparable to those obtained with scintigraphy”.(3) The previously observed offsets between γ-scintigraphy and breath test were assigned to differences in ^{13}C-metabolism. A linear transformation was proposed in order to achieve congruency of ^{13}C-breath test results and the two imaging techniques.(1, 5-8, 11)
Here, using MRI, such a linear relation common for all meals could not be identified. Neither the Bland-Altman plots (ref. **figure 1**) nor the linear regression analysis (ref. **figure 2**) detected any agreement with the MRI derived t_{50} values. As determined in the linear regression analysis, a linear transformation seems feasible only for test meals that do not show an emptying rate dependent offset of t_{50}, i.e., a regression slope of approximately 1.0, in the ^{13}C breath test data. The emptying rate dependency was less with the *ExpBeta* function and meal volume emptying when compared to total gastric content volume emptying. Together with the higher Pearson’s correlation

coefficients for all three nutrition formulas (r ranged 0.82 to 0.9) this may suggest that ^{13}C-acetate breath testing reflects meal volume rather than total gastric content volume (meal + secretion) emptying. Neither the Bland-Altman plots (ref. **figure 1**) nor the linear regression analysis (ref. **figure 2**) detected any agreement with the MRI derived t_{50} values. In the two enteral formulas NU and OS that did not show emptying rate dependent deviation of ^{13}C-breath test from MRI meal volume data, the ^{13}C-acetate breath test may be used in within-subject longitudinal studies when using the *ExpBeta* function for analysis.

The wider limits of agreement for BT_t_{50}_WN indicated inferior agreement with MRI compared to the t_{50} derived with the *ExpBeta* function .(20) In addition, the deviation of BT_t_{50}_WN from MRI values increased with longer t_{50} values (ref. **figures 1** and **2**). This important observation might explain some discrepancy to previous validation studies using γ-scintigraphy, in which t_{50} values were in the range of 35 to 45 minutes.(2, 3). Here, MRI derived t_{50} values were in the range of 80 and 110 minutes for MRI_t_{50}_MV and MRI_t_{50}_GCV, respectively (detailed data not presented). In consequence, the use of the *WN* method may only be valid for half-emptying times of meal and/or gastric content below the values observed in this MRI study.

In conclusion, this validation study indicates that the ^{13}C-acetate breath test may not be applied to compare gastric emptying of arbitrary liquid test meals without prior validation by imaging methods. Even for isocaloric and isovolumetric liquid meals, a linear transformation for t_{50} common for all enteral formulas could not be identified. Validation and/or normalization by a gastric content sensitive imaging modality is highly recommended to allow for the correct interpretation of ^{13}C-acetate derived gastric emptying data.

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Disclosures

No conflicts of interest exist.

Specific Author Contributions

SK performed the data acquisition, did statistical analyses and wrote the paper. DM provided the R code to analyze breath test data and analyzed the MRI data. AS, WS and MF designed and supervised the study. AS did statistical analyses and critically revised the manuscript. All authors have approved the final draft submitted.

References

1. Braden B, Adams S, Duan LP, Orth KH, Maul FD, Lembcke B, Hor G, Caspary WF. The [13C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. Gastroenterology. 1995 Apr;**108**(4):1048-55.
2. Sanaka M, Urita Y, Sugimoto M, Yamamoto T, Kuyama Y. Comparison between gastric scintigraphy and the [13C]-acetate breath test with Wagner-Nelson analysis in humans. Clinical and experimental pharmacology & physiology. 2006 Dec;**33**(12):1239-43.
3. Sanaka M, Nakada K, Nosaka C, Kuyama Y. The Wagner-Nelson method makes the [13C]-breath test comparable to radioscintigraphy in measuring gastric emptying of a solid/liquid mixed meal in humans. Clinical and experimental pharmacology & physiology. 2007 Jul;**34**(7):641-4.
4. Sanaka M, Yamamoto T, Ishii T, Kuyama Y. The Wagner-Nelson method can generate an accurate gastric emptying flow curve from CO2 data obtained by a 13C-labeled substrate breath test. Digestion. 2004;**69**(2):71-8.
5. Cappello G, Malatesta MG, Ferri A, Ciccaglione AF, Toracchio S, Grossi L, Marzio L. Gastric emptying of a solid-liquid meal measured with 13C octanoic acid breath test and real-time ultrasonography: a comparative study. The American journal of gastroenterology. 2000 Nov;**95**(11):3097-100.
6. Choi MG, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS. [13C]octanoic acid breath test for gastric emptying of solids: accuracy, reproducibility, and comparison with scintigraphy. Gastroenterology. 1997 Apr;**112**(4):1155-62.
7. Choi MG, Camilleri M, Burton DD, Zinsmeister AR, Forstrom LA, Nair KS. Reproducibility and simplification of 13C-octanoic acid breath test for gastric emptying of solids. The American journal of gastroenterology. 1998 Jan;**93**(1):92-8.
8. Lee JS, Camilleri M, Zinsmeister AR, Burton DD, Choi MG, Nair KS, Verlinden M. Toward office-based measurement of gastric emptying in symptomatic diabetics using [13C]octanoic acid breath test. The American journal of gastroenterology. 2000 Oct;**95**(10):2751-61.
9. Sanaka M, Yamamoto T, Kuyama Y. Theoretical flaws in the gastric emptying breath test: why is it dubious? Digestive diseases and sciences. 2005 Jan;**50**(1):15-7.
10. Sanaka M, Yamamoto T, Kuyama Y. Retention, fixation, and loss of the [13C] label: a review for the understanding of gastric emptying breath tests. Digestive diseases and sciences. 2008 Jul;**53**(7):1747-56.
11. Ghoos YF, Maes BD, Geypens BJ, Mys G, Hiele MI, Rutgeerts PJ, Vantrappen G. Measurement of gastric emptying rate of solids by means of a carbon-labeled octanoic acid breath test. Gastroenterology. 1993 Jun;**104**(6):1640-7.
12. Curcic J, Sauter M, Schwizer W, Fried M, Boesiger P, Steingoetter A. Validation of a golden angle radial sequence (GOLD) for abdominal T1 mapping during free breathing: Demonstrating clinical feasibility for quantifying gastric secretion and emptying. Journal of magnetic resonance imaging : JMRI. 2014 Jan 6.
13. Sauter M, Curcic J, Menne D, Goetze O, Fried M, Schwizer W, Steingoetter A. Measuring the interaction of meal and gastric secretion: a combined quantitative magnetic resonance imaging and pharmacokinetic modeling approach. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2012 Jul;**24**(7):632-8, e272-3.
14. Schwizer W, Steingoetter A, Fox M. Magnetic resonance imaging for the assessment of gastrointestinal function. Scandinavian journal of gastroenterology. 2006 Nov;**41**(11):1245-60.

15. Treier R, Steingoetter A, Goetze O, Fox M, Fried M, Schwizer W, Boesiger P. Fast and optimized T1 mapping technique for the noninvasive quantification of gastric secretion. Journal of magnetic resonance imaging : JMRI. 2008 Jul;**28**(1):96-102.
16. Kuyumcu S, Menne D, Curcic J, Goetze O, Klebach M, Abrahamse E, Hofman Z, Fried M, et al. A Noncoagulating Enteral Formula Can Empty Faster From the Stomach: A Double-Blind, Randomized Crossover Trial Using Magnetic Resonance Imaging. JPEN Journal of parenteral and enteral nutrition. 2014 Apr 3.
17. Kuyumcu S, Goetze O, Menne D, Treier R, Boesiger P, Fox M, Fried M, Schwizer W, et al. Gastric secretion does not affect the reliability of the 13C-acetate breath test: A validation of the 13C-acetate breath test by magnetic resonance imaging. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society. 2013 Feb;**25**(2):176-e87.
18. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: a height-weight formula validated in infants, children, and adults. The Journal of pediatrics. 1978 Jul;**93**(1):62-6.
19. Pinheiro JB, Douglas; DebRoy, Saikat; Sarkar, Deepayan; and the R Development Core Team nlme: Linear and Nonlinear Mixed Effects Models. R package version 31-1082013.
20. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. Lancet. 1986 Feb 8;**1**(8476):307-10.
21. Lin LI. A concordance correlation coefficient to evaluate reproducibility. Biometrics. 1989 Mar;**45**(1):255-68.
22. Malagelada JR, Robertson JS, Brown ML, Remington M, Duenes JA, Thomforde GM, Carryer PW. Intestinal transit of solid and liquid components of a meal in health. Gastroenterology. 1984 Dec;**87**(6):1255-63.
23. Goetze O, Steingoetter A, Menne D, van der Voort IR, Kwiatek MA, Boesiger P, Weishaupt D, Thumshirn M, et al. The effect of macronutrients on gastric volume responses and gastric emptying in humans: A magnetic resonance imaging study. American journal of physiology Gastrointestinal and liver physiology. 2007 Jan;**292**(1):G11-7.
24. Kwiatek MA, Menne D, Steingoetter A, Goetze O, Forras-Kaufman Z, Kaufman E, Fruehauf H, Boesiger P, et al. Effect of meal volume and calorie load on postprandial gastric function and emptying: studies under physiological conditions by combined fiber-optic pressure measurement and MRI. American journal of physiology Gastrointestinal and liver physiology. 2009 Nov;**297**(5):G894-901.
25. Franke A, Harder H, Singer MV. Reliability of the [13C]-acetate breath test in the measurement of gastric emptying of ethanol solutions: a methodological study. Scandinavian journal of gastroenterology. 2004 Aug;**39**(8):722-6.
26. Hellmig S, Von Schoning F, Gadow C, Katsoulis S, Hedderich J, Folsch UR, Stuber E. Gastric emptying time of fluids and solids in healthy subjects determined by 13C breath tests: influence of age, sex and body mass index. Journal of gastroenterology and hepatology. 2006 Dec;**21**(12):1832-8.
27. Achour L, Meance S, Briend A. Comparison of gastric emptying of a solid and a liquid nutritional rehabilitation food. European journal of clinical nutrition. 2001 Sep;**55**(9):769-72.
28. Sanggaard KM, Holst JJ, Rehfeld JF, Sandstrom B, Raben A, Tholstrup T. Different effects of whole milk and a fermented milk with the same fat and lactose content on gastric emptying and postprandial lipaemia, but not on glycaemic response and appetite. The British journal of nutrition. 2004 Sep;**92**(3):447-59.

29. Brun AC, Stordal K, Johannesdottir GB, Bentsen BS, Medhus AW. The effect of protein composition in liquid meals on gastric emptying rate in children with cerebral palsy. Clinical nutrition. 2012 Feb;**31**(1):108-12.
30. Savage K, Kritas S, Schwarzer A, Davidson G, Omari T. Whey- vs casein-based enteral formula and gastrointestinal function in children with cerebral palsy. JPEN Journal of parenteral and enteral nutrition. 2012 Jan;**36**(1 Suppl):118S-23S. Epub 2012/01/18.
31. van den Braak CC, Klebach M, Abrahamse E, Minor M, Hofman Z, Knol J, Ludwig T. A novel protein mixture containing vegetable proteins renders enteral nutrition products non-coagulating after in vitro gastric digestion. Clinical nutrition. 2013 Oct;**32**(5):765-71.

Tables

Table 1: Enteral formulas (16)

	FR[1]	NU[2]	OS[3]
Kcal*mL^{-1}	1.5	1.5	1.5
Protein [%]	15	16	16.7
Protein composition	80% casein, 20% whey	25% casein, 35% whey, 20% soy, 20% pea	84% casein, 16% soy
Fat [%]	35	35	29
Carbohydrate [%]	50	49	54.3
Osmolarity [mosm*L^{-1}]	330	360	390

[1]Fresubin Energy® (Fresenius Kabi, Bad Homburg, Germany) with a coagulating protein mix.
[2]Nutrison Energy® (Nutricia Advanced Medical Nutrition, Zoetermeer, the Netherlands) with a non-coagulating protein mix.
[3]Osmolite HiCal® (Abbott Nutrition, Baar, Switzerland) with a coagulating protein mix. (16)

Table 2: Biases and limits of agreement of the Bland-Altman plots depicted in figure 1. Values are given in minutes and as bias (95%CI) [lower limit of agreement (95%CI); upper limit of agreement (95%CI)].

	Agreement of BT_t_{50}_ExpBeta and		Agreement of BT_t_{50}_WN and	
	MRI_t_{50}_MV	**MRI_t_{50}_GCV**	**MRI_t_{50}_MV**	**MRI_t_{50}_GCV**
FR	48.0 (42 to 53) [26 (16 to 34); 71 (61 to 79)]	21.4 (13 to 29) [-13 (-27 to 1); 56 (41 to 70)]	-40.4 (-49 to -31) [-78 (-94 to -63); -2 (-18 to 13)]	-67.0 (-79 to -54) [-118 (-139 to -97); -16 (-37 to 5)]
NU	54.8 (49 to 60) [32 (23 to 41); 77 (68 to 86)]	25.7 (13 to 39) [-31 (-53 to -8); 82 (59 to 104)]	-36.0 (-41to-31) [-58 (-67 to-49); -14 (-23 to -4)]	-65.0(-80 to -50) [-129 (-155 to -103); -1.0 (-27 to 25)]
OS	56.3 (51 to 60) [38 (30 to 46); 74 (66 to 82)]	28.4 (20 to 37) [-4 (-18 to 10); 61 (47 to 75)]	-32.0 (-40 to -24) [-62 (-75 to-49); -2 (-15 to 11)]	-59.8 (-71 to -49) [-103 (-121 to -84); -17.0(-36 to 2)]

Table 3: Parameters of the linear regression for t_{50} values from breath test ($BT\_t_{50}\_ExpBeta$ and $BT\_t_{50}\_WN$) and MRI ($MRI\_t_{50}\_MV$ and $MRI\_t_{50}\_GCV$) per enteral formula. Given are the slopes, intercepts, Lin's concordance correlation coefficients (r_c) and Pearson's correlation coefficients (r), together with 95% CI of the linear regression equations displayed in **figure 2**.

	FR	NU	OS
$BT\_t_{50}\_ExpBeta$ vs. $MRI\_t_{50}\_MV$			
Slope	0.7 (0.5 to 0.9)	1.3 (1.0 to 1.6)	1.0 (0.7 to 1.3)
y-intercept	72.8 (52.8 to 92.9)	32.8 (8.7 to 56.8)	56.3 (34.2 to 78.4)
r_c	0.18 (0.07 to 0.29)	0.17 (0.07 to 0.27)	0.14 (0.05 to 0.24)
r	0.82 (0.59 to 0.92)	0.90 (0.76 to 0.96)	0.88 (0.7 to 0.95)
$BT\_t_{50}\_WN$ vs. $MRI\_t_{50}\_MV$			
Slope	0.2 (-0.1 to 0.5)	0.4 (0.2 to 0.6)	0.3 (0.0 to 0.5)
y-intercept	22.5 (-1.7 to 46.7)	6.7 (-9.2 to 22.5)	24.1 (3.8 to 44.4)
r_c	0.08 (-0.03 to 0.18)	0.14 (0.04 to 0.23)	0.1 (-0.01 to 0.21)
r	0.36 (-0.1 to 0.69)	0.72 (0.43 to 0.88)	0.46 (-0.01 to 0.76)
$BT\_t_{50}\_ExpBeta$ vs. $MRI\_t_{50}\_GCV$			
Slope	0.5 (0.3 to 0.7)	0.4(0.2 to 0.6)	0.6 (0.3 to 0.9)
y-intercept	76.6 (55.4 to 97.9)	88.4(62.7 to 114.0)	71.7 (41.7 to 102.0)
r_c	0.48 (0.24 to 0.66)	0.43 (0.16 to 0.64)	0.38 (0.14 to 0.58)
r	0.78 (0.52 to 0.91)	0.65 (0.3 to 0.84)	0.74 (0.41 to 0.9)
$BT\_t_{50}\_WN$ vs. $MRI\_t_{50}\_GCV$			
Slope	0.2 (-0.1 to 0.4)	0.2 (0.1 to 0.3)	0.2 (-0.0 to 0.4)

y-intercept	24.8 (0.8 to 48.8)	23.8 (12.3 to 35.3)	25.2 (5.8 to 44.6)
r_c	0.04 (-0.02 to 0.1)	0.08 (0.01 to 0.14)	0.05 (-0.01 to 0.1)
r	0.32 (-0.14 to 0.67)	0.60 (0.22 to 0.81)	0.46 (-0.01 to 0.76)

Figure legends

Figure 1: Bland-Altman plots of t_{50} values from breath test (BT_t_{50}_ExpBeta and BT_t_{50}_WN) and MRI (MRI_t_{50}_MV and MRI_t_{50}_GCV) for all enteral formulas. Values of bias and limits of agreement are given in **table 2**.

Figure 2: Linear regression for t_{50} values from breath test and MRI per enteral formula. Displayed are the linear regressions (solid lines) with lower and upper 95%CI between BT_t_{50}_ExpBeta (grey shaded) and BT_t_{50}_WN (black shaded) and (**A**) MRI_t_{50}_MV or (**B**) MRI_t_{50}_GCV, respectively. Axes are iso-scaled, so that the dashed line has a slope of 1.0. Details on values of the linear regression lines are displayed in **table 3**.

Supplementary Figure S1: Individual PDR for the three enteral formulas, displayed with red (NU), blue (FR) and green (OS) lines and dots. Raw data is displayed by dots with the related modelled PDR curve overlaid in a line with corresponding colour.

Supplementary Figure S2: Individual meal volume curves over time for the three enteral formulas displayed with red (NU), blue (FR) and green (OS) lines.